Bone Regeneration and Docking Site Healing After Bone Transport Distraction Osteogenesis in the Canine Mandible

Lucy K. Nagashima, DDS, Michelle Rondon-Newby, DDS,†*
Ibrahim E. Zakhary, DDS,‡ William W. Nagy, DDS,§
Uriel Zapata, MSc, PhD,‖ Paul C. Dechow, PhD,¶
Lynne A. Opperman, PhD,# and
*Mohammed E. Elsalanty, MD, PhD***

Purpose: Bone transport distraction osteogenesis provides a promising alternative to traditional grafting techniques. However, existing bone transport distraction osteogenesis devices have many limitations. The purpose of this research was to test a new device, the mandibular bone transport reconstruction plate, in an animal model with comparable mandible size to humans and to histologically and mechanically examine the regenerate bone.

Materials and Methods: Eleven adult foxhounds were divided into an unreconstructed control group of 5 animals and an experimental group of 6 animals. In each animal, a 34-mm segmental defect was created in the mandible. The defect was reconstructed with a bone transport reconstruction plate. Histologic and biomechanical characteristics of the regenerate and unrepaired defect were analyzed and compared with bone on the contralateral side of the mandible after 4 weeks of consolidation.

Results: The reconstructed defect was bridged with new bone, with little bone in the control defect. Regenerate density and microhardness were 22.3% and 42.6%, respectively, lower than the contralateral normal bone. Likewise, the anisotropy of the experimental group was statistically lower than in the contralateral bone. Half the experimental animals showed nonunion at the docking site.

Conclusion: The device was very stable and easy to install and activate. After 1 month of consolidation, the defect was bridged with new bone, with evidence of active bone formation. Regenerate bone was less mature than the control bone. Studies are underway to identify when the regenerate properties compare with normal bone and to identify methods to augment bone union at the docking site.

J Oral Maxillofac Surg 70:429-439, 2012

Segmental defects of the mandibular bone are difficult to reconstruct. These defects may result from trauma, benign and malignant tumor resections, and infections. Consequences include facial deformities, impaired masticatory performance, lip incompetence, poor speech functions, and impaired deglutition.[1]

Bone transport distraction osteogenesis (BTDO) is a method of bone defect reconstruction that has been

*Resident and MSc Student, Department of Prosthodontics, Baylor College of Dentistry, Texas A&M University System, Dallas, TX.

†Resident and MSc Student, Department of Prosthodontics, Baylor College of Dentistry, Texas A&M University System, Dallas, TX.

‡Postdoctoral Fellow, Department of Oral Biology, College of Dental Medicine, Georgia Health Science University, Augusta, GA.

§Professor, Department of Prosthodontics, Baylor College of Dentistry, Texas A&M University System, Dallas, TX.

‖Postdoctoral Fellow, Department of Biomedical Sciences, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX; Currently at Mechanical Engineering Department, Eafit University, Medellín, Colombia.

¶Professor, Department of Biomedical Sciences, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX.

#Professor, Department of Biomedical Sciences, Texas A&M Health Science Center, Baylor College of Dentistry, Dallas, TX.

**Assistant Professor, Department of Oral Biology, College of Dental Medicine, Georgia Health Science University, Augusta, GA.

Drs. Opperman and Elsalanty both have Company Ownership and Patent Ownership in Craniotech.

Address correspondence and reprint requests to Dr Elsalanty: Departments of Oral Biology and Oral and Maxillofacial Surgery, College of Dental Medicine, Georgia Health Science University, 1120 15th St, Augusta, GA 30912; e-mail: melsalanty@georgiahealth.edu

0278-2391/12/7002-0$36.00/0
doi:10.1016/j.joms.2011.02.016

used in the long bones for decades. Using the principles of distraction osteogenesis, BTDO works by creating an osteotomy by detaching a bone segment (transport disc) from 1 end of the defect and moving it gradually across the gap to the other end. As the bone segment is moved, bone should form behind it, filling the defect. The new bone should have similar quality and physical dimensions to the original bone.[2]

Several bone transport distraction devices are available for mandibular reconstruction, with very promising results. Some of these devices can be applied extraorally through transcutaneous pins,[3-5] and others are buried under the mucosa.[6-11] However, current devices have many limitations that mitigate their use on a wide scale.

In a canine segmental mandibular defect model, a new intraoral bone transport device, the bone transport reconstruction plate (BTRP), was tested.[12] In this device, the reconstruction plate itself functioned as the transport track, eliminating the need to have a superimposed device that carries the transport track. This has remarkably decreased the size of the device and facilitated its installation, activation, and adjustment procedures. It also allowed for staging the procedure, because the device can be retained for a long time with minimal discomfort to the patient. It allowed crossing the midline for anterior mandibular defects, because it can be retained for prolonged periods under the mucosa. The present study analyzed the histologic and biomechanical parameters of new bone created within a unilateral segmental mandibular defect compared with normal bone of the contralateral side of the mandible. The advantages, disadvantages, and limitations of the device and technique are emphasized.

FIGURE 1. Bone transport reconstruction plate. *A,* The device is attached to a cadaver dog mandible in which a 35-mm defect has been created. The transport disc is shown attached to the transport unit of the device with microscrews and mobilized across the defect. *B,* Transport unit of the device.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

Materials and Methods

ANIMALS

Eleven adult foxhounds weighing 81.5 ± 8.3 lb were divided into an untreated control group of 5 animals and an experimental group of 6 animals. In the 2 groups, a unilateral segmental defect 33.8 ± 8.4 mm long was created. Each defect was stabilized by the plate portion of the BTRP (Craniotech ACR Devices, LLC, Dallas, TX; Fig 1). In the experimental group, the transport unit of the device was installed on the plate and the defect was reconstructed by the BTRP. In the control group, the defect was not reconstructed further. At autopsy examination, the mandibles were collected and the devices were removed. Bone regenerates were harvested, fixed, and processed for undecalcified histologic sections. Bone mechanical properties were tested in the regenerate using microindentation and ultrasound. Comparisons were made between the bone regenerate and the contralateral (nonoperated) sides of the mandible.

The untreated control animals were used to confirm the size of the defect as a critical-size defect in this model. Based on previous experience with critical-size defect models, an untreated control group was necessary to confirm that a similar-size defect would not heal spontaneously with the duration and method of bone fixation and housing and nutritional conditions of the experimental animals. The housing, care, and experimental protocol were in accordance with the guidelines set forth by the Baylor College of Dentistry institutional animal care and use committee.

DEVICE AND SURGICAL PROTOCOL

Preoperatively, the mandibular canine tooth on the surgical side had root-canal treatment in anticipation of root injury by the fixation screws. The maxillary third and fourth premolars and the first molar on the

same side of the defect were extracted to eliminate occlusal trauma to the mucosa covering the defect zone.

Device description, installation, and surgical protocols used in this experiment have been previously published.[12] Briefly, a segmental (full-thickness) distraction gap of approximately 35 mm was created so the bone was removed with its periosteal sleeve. The defect was stabilized by the reconstruction plate component of the BTRP fixed with 3 2.7-mm titanium screws on each side. In the experimental animals, a transport segment of approximately 1.5 cm in width was separated from the proximal mandibular stump by a bicortical osteotomy. The transport unit of the device was installed on the middle segment of the plate so the activation screw could engage the threads on the transport track. The transport unit was fixed to the transport bone segment with at least 4 1.7-mm titanium mini-screws. The activation cable was extruded posteriorly through a skin incision behind the angle of the mandible.

The animals were monitored by observation of general attitude, posture, eating, watering of the eyes, salivating, sensitivity to touch, tongue manipulation, and rubbing and pawing of the mouth. Activation of the BTRP commenced after a latency period of 5 days and progressed at a rate of 0.5 mm/12 hours (morning and evening), unless accelerated consolidation occurred, which was perceived manually as difficulty in turning the activation screw. In this case, the distraction rate was accelerated to 0.75 mm twice daily. Active bone transport distraction continued for 4 to 6 weeks until the transport segment reached the opposite edge of the defect, the docking site. This was followed by a consolidation period of 4 weeks before euthanasia using a protocol described previously.[12] Untreated control animals were sacrificed 10 weeks after surgery. The mandibles were collected for analysis, and the devices were removed.

HISTOLOGIC ANALYSIS

The mandibles were resected en bloc, sectioned into right and left halves, and placed in 10% formaldehyde. Each hemi-jaw (regenerate and contralateral bone in the experimental group and untreated defect in the control group) was cut into individual blocks. The size of the blocks was approximately 35 mm in length by 25 mm in height by 10 mm in width. The blocks were processed for undecalcified histologic sections, 2 to 4 sections per block.

Specimens were embedded in methylmethacrylate and serial sagittal sections were prepared at thicknesses from 100 to 120 μm. Sections were ground, polished to approximately 75 μm, and stained with Stevenel blue and Van Giesen picro-fuchsin for histologic examination. The undecalcified bone samples were photographed with a digital camera (Canon Rebel XT; CanonUSA Inc, New York, NY) at a magnification of $\times$1.6. Measurements of all slides were taken using NIS-Element Advanced Research 2.30 (Nikon Software, Melville, NY) that was calibrated (1 pixel = 1 μm). In the untreated control defect and regenerate of the experimental animal slides, the amount of bone was quantified as the percentage of bone in the defect relative to the total area of the defect represented on each slide. In sections of the docking site, the presence or absence of bone union was described.

MECHANICAL TESTING

Specimen Preparation

A line parallel to the lower border of the mandible was drawn at the level where the specimens were harvested. This line marked the orientation of the excised discs and would be used to align the discs during the ultrasound experiment to identify the axis of maximum stiffness. One disc 5 mm in diameter from each bone specimen was cut from the buccal plate near the lower border of the mandible with a trephine bur under constant irrigation with a saline solution. The specimens were stored in a 10% buffered solution of formalin in a sealed container. These were kept in this container throughout the preparation process until testing (n = 5 regenerate; n = 5 host). Any trabecular bone present on the specimen was removed with a fine-grain grinding wheel. The diameter and thickness of each specimen was measured with a digital caliper.

Density Measurement

The apparent density of specimens (milligrams per cubic centimeter) was calculated according to Archimedes' principle of buoyancy.[13] Each specimen was weighed while suspended in distilled water. The specimens were then dried by lightly patting with absorbing paper to ensure that only the internal water remained. The specimens were then weighed again (M). These procedures were repeated 3 times to verify accuracy. The density of the specimens was calculated from the equation: $\rho = (M\rho w)/(M - S)$, where ρw is the density of the water, and S is the weight of the specimen suspended in distilled water.

Ultrasonic Measurements of Material Properties

The ultrasonic velocities were measured with a pulse transmission technique described previously.[13-16] In brief, longitudinal ultrasonic waves were generated by Panametrics V312-N-SU transducers (Olympus NDT, Houston, TX) resonating at 10 MHz. The transducers were powered with a Hewlett-Packard Model 214 A pulse generator (Hewlett-Packard Palo Alto, CA). Pulse delays induced by passage of ultrasonic

waves through the bone were read on a Tektronix TDS420 digitizing oscilloscope (Tektronix Texas LLC, Richardson, TX). The bone cylinder was mounted on a 4-inch Rotary Table P/N 3700 (Sherline Products, Inc, Vista, CA), which allowed accurate rotations for a 360° turn. Ultrasonic velocities were calculated by dividing the specimen thickness or diameter by the recorded time delay minus the standard system delay.[13] The measurement was taken in 18 different directions by rotating the specimens by 10° after each measurement.

A refined method of determining the axes of minimum and maximum stiffness in the plane of the cortical plate was used. A program written in Mathcad 2001 (Mathsoft Engineering and Education, Inc, Cambridge, MA) was used to fit the calculated longitudinal velocities and their angular orientation for each bone specimen to a sine function ($a \times \sin[X + b] + c$). The coefficients a, b, and c corresponded to the orientation of the axes of maximum stiffness, the average velocity, and the maximum deviation of the curve from the average velocity. The direction of the axis of maximum stiffness corresponded to the direction of peak longitudinal velocity. Likewise, the minimal principal axis, or least stiffness direction, corresponded to the lowest velocity. The third axis was always tangential or perpendicular to the cortical plane. Anisotropy was calculated by dividing minimum velocity by maximum velocity.

Microindentation Measurement

Material hardness is defined as its resistance to penetration by a solid body.[17] Indentation tests measure bone hardness by driving an indenter with a known geometry into a bone section. The Knoop indenter has a rhombic-shaped pyramidal diamond tip (Fig 2). Specimens were mounted in resin blocks and smoothed with carborundum paper of increasing fineness from 240 to 600 grit. Two vertical lines perpendicular to each other were drawn onto the surface of the polished bone specimen to evenly distribute 4 microindentations. The mounted specimens were secured in a leveling vice of the microhardness testing machine, allowing the indenter to penetrate the specimen at a right angle. The testing mechanism was mounted to be free of vibration. Using the Knoop indenter, an indenting load of 25 g was used with a descent time of 15 seconds. Upon reaching the lowest position, the diamond was allowed to impress the material for an additional dwell time of 10 seconds before being released. The average of the 2 diagonal lengths for each indentation was automatically calculated and the Knoop microhardness number was provided by the formula: $HK = P/A = P/CL^2$, where P is the applied load in kilograms, A is the projected area of indentation (square millimeters), L is the measured length (millimeters) of the long diagonal, and C (0.07028) is a constant for the indenter relating the projected area to the square of the length of the long diagonal.[17]

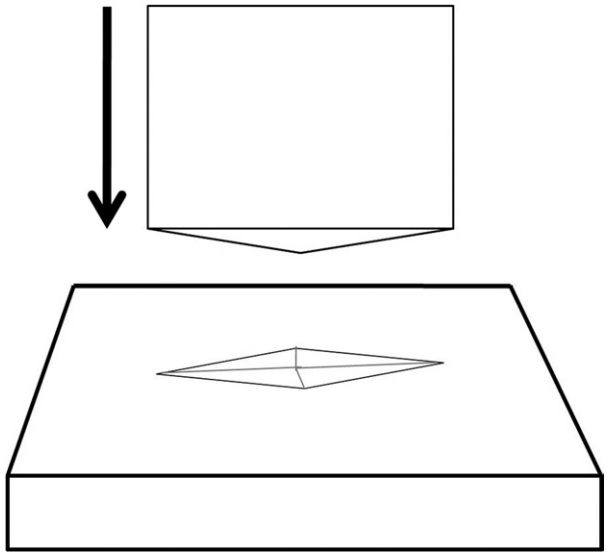

FIGURE 2. Knoop indentation of the bone surface. A rhombic-shaped pyramidal diamond tip is introduced into the bone surface under 25 g of force. Microhardness of bone is calculated based on dimensions of the resulting indent, because these define how much the diamond tip penetrated the surface.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

STATISTICAL ANALYSIS

For statistical evaluation of histomorphometric data, a 2-sample t test was used for comparisons between the experimental and nontreated control animals. A Kruskal-Wallis test was used for testing equality of population medians between groups. The tests assumed a normally shaped and scaled distribution for each group.

Biomechanical results are displayed as means ± standard deviations unless otherwise stated. A 2-sample t test and a Kruskal-Wallis test were performed to evaluate the total percentage of bone volume formed in the defects of the 2 groups. Minitab 14 (Minitab, State College, PA) was used to compare thickness, diameter, hardness, and ultrasonic measurements in regenerate versus host bone using paired t test statistical analysis. Differences were considered statistically significant at $P < .05$. The orientation of maximum stiffness during ultrasonic measurements was determined using Mathcad 2001 (Mathsoft Engineering and Education, Inc) and assessed using circular statistical software (Oriana, Kovach Computing Services, Anglesey, Wales, UK).

Results

Most dogs tolerated the surgical and postoperative procedures well, without any major complications. Soft diet and normal activities resumed immediately after surgery. Two dogs showed loosening of the transport device screw and underwent a surgical procedure to correct it. One animal was excluded from the study because of complications from wound dehiscence. The remaining lengthening period occurred without significant incident.

HISTOLOGIC AND HISTOMORPHOMETRIC EVALUATIONS

Tissues from 1 animal in the untreated control group were excluded because the edges of the defect could not be identified. Control animals showed no sign of bone regeneration across the surgical defect. The defect contained mostly fibrous tissue in the middle of the defect, with very small areas of regenerate bone formed from the edges of the host bone into the defect. This small amount of calcified tissue appeared woven and lacked evidence of maturation (Fig 3A,B).

All animals in the experimental group showed remarkable bone regeneration across the surgical defect. Bone formed from the host bone toward the transport disc and from the transport disc back to the host bone, producing less well-developed bone in the center of the defect and thicker bone at the outer edges of the defect (Fig 3C,D). The bone volume fraction in the repaired defect was 43% in the experimental animals versus 6% in the untreated control group ($P < .01$; Fig 4).

Half the dogs showed nonunion at the docking site. Histologic sections from the docking sites in all animals showed that the transport disc produced a cone-shaped outgrowth of new bone into the anterior portion of the defect. The recipient bone segment also produced a conical outgrowth of new bone into the defect. As the transport segment approached, these cone-shaped bone outgrowths prevented the transport disc from having proper apposition with the host bone, giving the docking site an hourglass outline. In dogs where nonunion was observed, this pattern was associated with fibrous tissue interposition between the transport disc and the opposite segment of the defect, preventing any contact between the transport disc and the recipient mandibular bone segment (Fig 3E,F).

PHYSICAL PROPERTIES

Specimens prepared for mechanical testing were comparable in physical dimensions between the regenerate and contralateral sides. The regenerate specimens had a mean diameter of 4.91 ± 0.08 mm, whereas the contralateral specimens had a mean diameter of 4.77 ± 0.23 mm ($P > .05$). Regarding thickness, the contralateral samples had a mean thickness of 2.01 ± 0.40 mm, whereas the regenerate group had a mean thickness of 2.13 ± 0.32 mm. Differences in physical dimensions were not statistically significant (Table 1).

The regenerate samples had a mean density of 1,513 ± 171.7 kg/m^3, whereas the contralateral samples had a mean density of 1,947 ± 68.8 kg/m^3, a 22.3% statistically significant difference between the 2 groups (Table 1). The regenerate samples had a mean microhardness of 14.8 ± 2.0 kg/mm^2 that was statistically significantly lower than the contralateral samples with a mean microhardness of 25.8 ± 5.4 kg/mm^2 (Table 1).

The correlation coefficients (*rr*) were statistically significantly lower for the regenerate samples (0.62 ± 0.29) than for the contralateral samples (0.93 ± 0.09; Table 2). The angle of maximum stiffness was not significantly different between the 2 groups (regenerate, 33.3 ± 46.7; contralateral, 21.4 ± 35.3; Table 2) and coincided with a plane parallel to the lower border of the mandible. The maximum velocity (representing maximum stiffness) was statistically significantly higher for the contralateral samples (3.98 ± 0.22) compared with the regenerate group (3.3 ± 0.24; Table 2). The minimum velocity (representing minimum stiffness) did not show any statistically significant differences between groups (contralateral, 3.09 ± 0.37; regenerate, 3.04 ± 0.12; Table 2). The anisotropy (representing the difference in bone tissue orientation in different planes, a parameter of bone maturation) of the regenerate specimens (0.92 ± 0.06) was statistically significantly less than that of the contralateral group (0.77 ± 0.07; Table 2).

To determine correlations between microhardness and density and between anisotropy and density, paired *t* test showed a linear correlation between density and both microhardness and anisotropy. With an increase in density, an increase in microhardness (Fig 5A) and a more significant anisotropy were observed (Fig 5B).

Discussion

In this study, a new device, the BTRP, was tested in the reconstruction of mandibular segmental defects. The device proved to be easy to install, activate, and adjust. It was well tolerated by most animals. The advantage of BTRP is that it uses the middle segment of the reconstruction plate as the transport track. Therefore, the transport followed the contour of the original mandibular outline and was stable throughout the process. Success of the linear version of this device opens the way to testing the next phase, which is carrying the transport disc across the midline

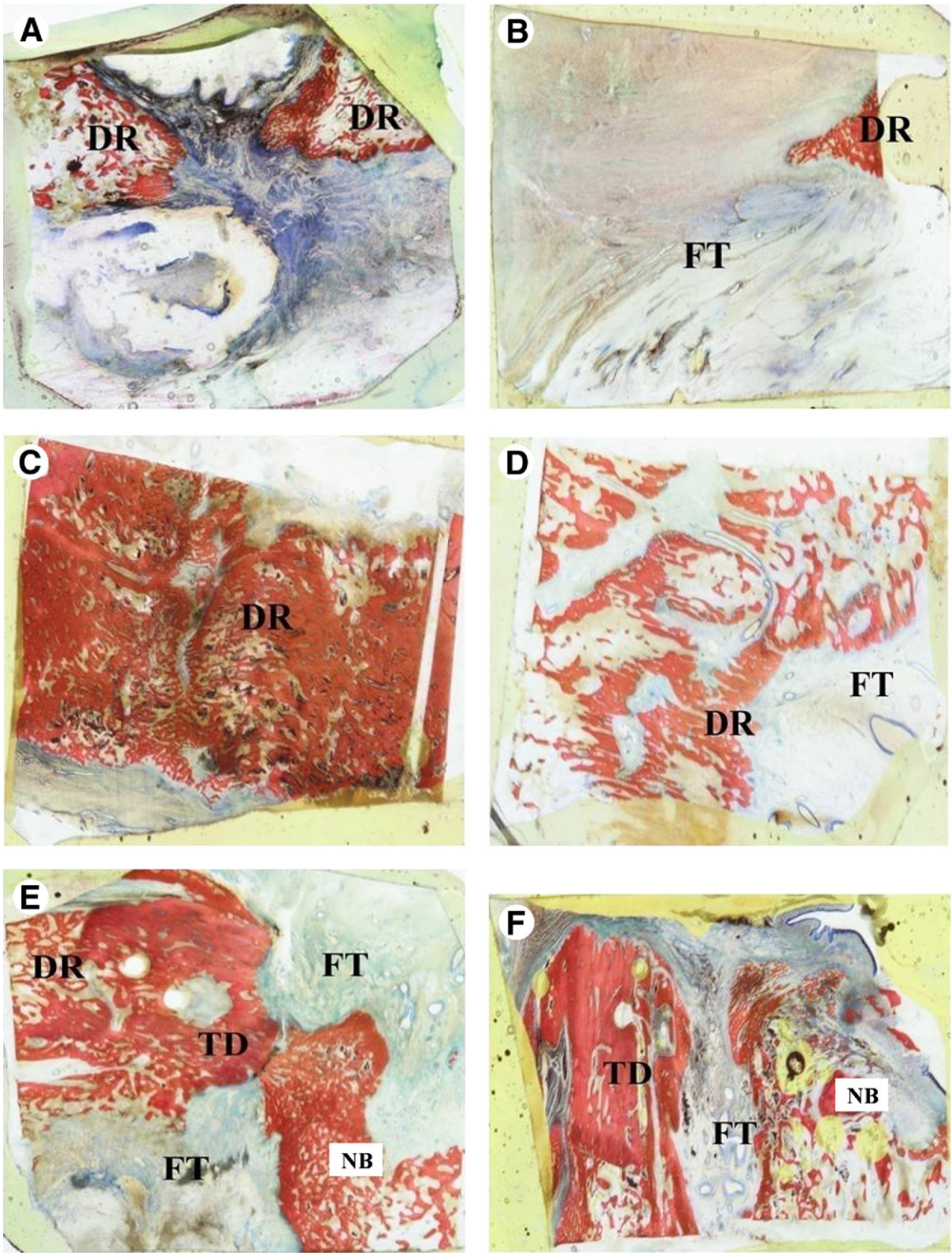

FIGURE 3. Histology of sagittal sections through the middle of new bone regenerate and docking site; undecalcified sections were stained with Stevenel blue and Van Giesen picro-fuchsin and photographed at ×1.6 magnification. *A,* Maximum bone regenerate observed in control group. *B,* Minimum bone regenerate in the control group. *C,* Maximum bone regenerate in the experimental group. *D,* Minimum bone formation in experimental group. *E,* Union at docking site (50% of experimental animals). *F,* Nonunion at docking site (50% of experimental animals). DR, distraction regenerate; FT, fibrous tissue; NB, new bone emerging from recipient bone segment; TD, transport disc.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

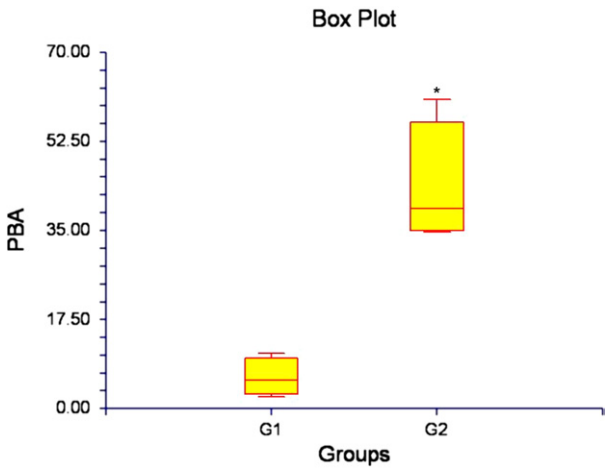

FIGURE 4. Difference in percentage of bone area (*PBA*) between control defects (*G1*) and reconstructed defects (*G2*). *Statistically significant at $P = .01$.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

along a curvilinear vector. Because the transport unit is small and is carried on the plate itself, it should be easy to delay the distraction process or divide it into stages, if needed, a unique advantage over previous devices.

Bone regeneration by the BTRP was successful, creating 43% fill of the defect after 1 month. Previous histologic studies examining bone regenerate of BTDO produced similar results at a later time point, 8 weeks of consolidation.[18-20] Zhao[21] reported that after 8 weeks of consolidation, the regenerated bone showed very thin bone trabeculae parallel to the distraction vector and there were signs of lamellar ossification. It was not until the 12th week of consolidation that the regenerate bone was thicker than the host bone and a more mature lamellar ossification was observed.[21]

Reconstruction of mandibular defects aims to restore normal jaw functions and maintain acceptable facial esthetics. Functional reconstruction requires building new bone within the defect with sufficient alveolar height and thickness and optimal architectural quality. This would allow for permanent restoration of dentition, maxillomandibular occlusion, mandibular continuity, and rehabilitation of jaw functions, such as mastication, deglutition, sensitivity of the mucosa, tongue movements, lip competence, and speech. At the same time, esthetic reconstruction requires the restoration of normal appearance of the soft tissues, facial symmetry, dental arch, and lower facial dimensions.[22-24]

Extraoral bone transport devices have the advantage of fast application, minimal interference with soft tissues, which is a major advantage whenever soft tissue flaps are used, and easy troubleshooting. However, these devices provide little torque to drive the transport disc against significant soft tissue resistance. Likewise, they provide minimal support to the transport disc against the tension forces of the soft tissue, resulting in significant inward displacement of the regenerate.[2]

Intraoral bone transport devices, in contrast, provide better stability to the bone segments, because these depend on reconstruction plates to stabilize the mandible. However, available devices are bulky and difficult to install and troubleshoot. Most importantly, these devices are limited by design to unilateral defects.[25]

Other investigators performed transport distraction in a smaller (20-mm) defect in dogs, allowing a period of 90 days for consolidation. Under their protocol, they achieved complete bone regeneration, meaning that the regenerated segment was similar in density and histologically compared with the rest of the mandible.[10]

It is essential to validate that the size of the defect is large enough to be called a critical-size defect whenever an animal model is used. The critical size for segmental defects in the dog mandible depends on the size and age of the animal, the preservation or excision of periosteum around the defect, rigidity of fixation, viability of the tissues around the defect, and the incidence of local complications after resection.

Table 1. MEANS AND STANDARD DEVIATIONS OF REGENERATE AND CONTRALATERAL (HOST) BONE SPECIMEN DIAMETER, THICKNESS, DENSITY, MICROHARDNESS, AND ANISOTROPY

	Diameter (mm)		Thickness (mm)		Density (kg/m^3)*		Microhardness (kg/mm^2)*		Anisotropy*	
Group	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Regenerate	4.91	0.08	2.13	0.32	1513	172	14.8	2.0	0.92	0.06
Contralateral (host)	4.77	0.23	2.01	0.40	1947	69	25.8	5.4	0.77	0.07
P	NS		NS		.003		.018		.042	

Abbreviations: NS, not significant; SD, standard deviation.
*Significant difference at $P < .05$.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

Table 2. ULTRASOUND MEASUREMENTS

Group	Correlation Coefficient		Angle of Maximum Stiffness*		Maximum Velocity†		Minimum Velocity		Anisotropy†	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Regenerate	0.62	0.29	33.3	46.7	3.30	0.24	3.04	0.11	0.92	0.06
Contralateral (host)	0.93	0.09	21.4	35.3	3.98	0.22	3.09	0.37	0.77	0.07
P	.04		NS		.01		NS		.042	

Abbreviations: NS, not significant; SD, standard deviation.
*Angle between the axis of maximum stiffness and the reference line, which is parallel to the lower mandibular border.
†Significant difference at $P < .05$.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

Other factors may also play a role in determining the generalized healing status of the animal, such as nutrition, physical activity, and general condition of the animal. Because of all these variables, it was important to include a control group of the same size and living conditions to validate this critical-size defect model. Huh et al[26] suggested that a 15-mm defect with excision of periosteum would not spontaneously heal during the dog's natural life. Other investigators suggested larger defects (30 mm).[27] In this model, we found that a 34-mm segmental mandibular defect with excision of overlying periosteum would not heal spontaneously in dogs under the present study conditions.

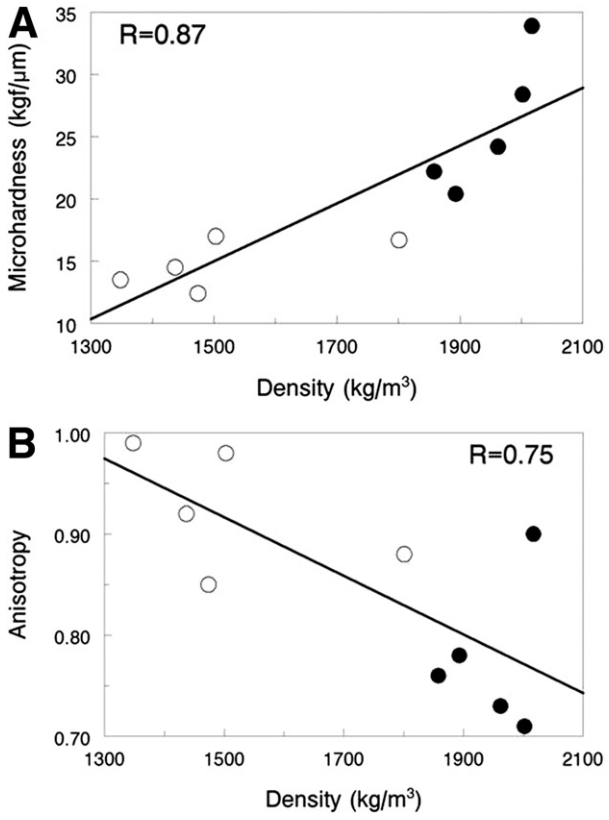

FIGURE 5. *A,* Correlation between microhardness and density comparing regenerate (*open circles*) with host (*solid circles*) bone specimens. *B,* Correlation between anisotropy and density comparing regenerate (*open circles*) with host (*solid circles*) bone specimens.

Nagashima et al. Quality of Distraction-Induced Regenerate. J Oral Maxillofac Surg 2012.

We previously showed that the physical dimensions of the regenerate at this time point are comparable to those of the contralateral bone, an important requirement for implant placement.[12] However, differences in density and microhardness values between the regenerate and contralateral bone presented in this study suggest that this time point would probably be too soon for implant placement. Further studies are underway to determine the time point most suitable for implant placement in the regenerate and transport disc.

Although radiographic and histological analyses of distracted bone are plentiful, few studies have examined its mechanical properties. In addition, the methodology used in various studies is highly variable, making results difficult to compare. Despite these differences, some studies have shown that the regenerate bone's mechanical properties are lower compared with host bone,[28-37] supporting the present microhardness results. However, 1 important issue of previous studies is the assumption that cortical bone can be modeled as an isotropic material, which is partly supported by 1 report[38] but contested by others.[16,39,40] The present study attempted to evaluate the regenerate bone at the microstructural level (osteons and haversian system) through microindentation testing, eliminating errors associated with whole-bone evaluations, and to assess the anisotropic characteristic of bone using ultrasonic longitudinal velocities.

The axis of highest velocity represents the axis of maximum stiffness, because ultrasound passes more rapidly as stiffness increases. The axis of minimum

stiffness corresponds to the axis of lowest velocity.[16] In the experimental group, the contralateral side showed increased maximum velocities and hardness compared to the regenerate.

The orientation at maximum longitudinal ultrasonic velocity was, in most specimens, close to the plane parallel to the lower border of the mandible for the regenerate and contralateral bone. This is similar to a previous study that showed that the inferior corpus of the human mandible had the least variation in the direction of maximum stiffness and was typically parallel to the lower border of the mandible.[40]

Degree of anisotropy was calculated using maximum and minimum velocities of longitudinal ultrasonic waves. The significantly less anisotropy observed in the regenerate group suggests less organization of bone in these specimens at 4 weeks of consolidation. A previous study reported the formation of immature woven bone with extracellular matrix relatively organized at 4 weeks of consolidation.[41] In contrast, another study described that during distraction-induced callus formation, collagen fibers were aligned parallel to the vector of distraction, suggesting that the subsequent mineralization would be in the same orientation as the distraction vector.[42] This, however, would relate more to the orientation of the early trabecular bone. At 4 weeks of consolidation, cortical condensation starts, from which the examined discs are harvested. The ultrasound data would, therefore, relate to the orientation of collagen fibers within the cortex and not the gross orientation of trabecular bone within the whole regenerate.

Hardness is the resistance of a material to penetration,[43,44] whereas stiffness relates to the amount of deformation of a material relative to the load applied to it.[16] In the present study, hardness was chosen to characterize the regenerate bone at the microscopic level. Specimens were preserved in formalin, which has unknown effects on the hardness of bone.[43,45,46] However, because the regenerate and contralateral bone samples underwent the same fixation protocols and duration, comparisons should not be influenced by the method of fixation.

Alternative testing methods for bone mechanical properties include compression/tension loading,[28] strain gauge measurement of bone stiffness,[29] 3-point bending,[31,47] cantilever bending,[30] and 4-point bending.[33] In the present study, the bone was examined at a relatively early time point (1 month of consolidation). Therefore, whole-bone mechanical tests would be inappropriate, because these do not account for anisotropic and inhomogeneous properties of the regenerate at this stage.[13,48]

BTDO has proved to be very successful in the reconstruction of segmental mandibular defects and in the generation of new bone in studies performed in animal models and in human patients.[4,7,12,49,50] Compared with other methods of mandibular reconstruction, including bone grafts, whether vascularized or nonvascularized, allogeneic materials, or guided-bone regeneration, BTDO offers the advantage of creating new bone that has the same physical dimensions and tissue properties as the host bone. In addition, BTDO eliminates donor site morbidity and shortens the surgical procedure.[10]

The most challenging aspect of transport distraction osteogenesis remains the achievement of union at the docking site. Nonunion was evident in half the animals. Some techniques were developed clinically to achieve union at the docking site, including sustained compression, alternate compression-distraction, bone grafting, and adjunctive therapies such as electromagnetic stimulation, low-intensity ultrasound, and bone-inducing factors.[51,52]

For the success of these techniques, it is very important that the transport disc maintains an adequate blood supply. This is important for bone union at the docking site and for a more rapid consolidation.[53,54] Also, the transport disc must remain stable and attached to the transport unit and must have adequate apposition with the distal native bone stump. The distraction device must also have the mechanical properties to hold the transport segment firmly at the docking site and preferably be able to apply sustained compression after docking.[8] Two more issues interfere with healing at the docking site. First is the growth of bone spikes from the recipient bone segment that prevent the advancement of the transport segment all the way to the docking site. Second is the interposition of soft tissues between the advancing edge of the transport disc and the recipient bone segment. These 2 issues were encountered within the present experimental group.

It is possible to reconstruct segmental mandibular defect using a BTRP. At 4 weeks of consolidation, all experimental defects showed successful defect bridging, with 46% of bone volume restored within the defect and 50% of union achieved at the docking site. At this time point, the regenerate bone has not yet achieved full mechanical integrity, evident by lower density, lower microhardness values, and less significant anisotropy values compared with the host bone. The device proved stable and easy to use and the results are promising for reconstruction of large mandibular defects. More studies are underway to test the appropriate conditions for implant placement, the possibility of reconstructing bone across the midline, and methods to improve healing at the docking site.

Acknowledgments

This research is supported by phase I and II STTR grants R41 DE15437-01 and R42 DE015437-02 from the National Institute of Dental and Craniofacial Research.

References

1. Olvera-Caballero C: Mandibular reconstruction in children. Microsurgery 20:158, 2000
2. Elsalanty ME, Taher TN, Zakhary IE, et al: Reconstruction of large mandibular bone and soft-tissue defect using bone transport distraction osteogenesis. J Craniofac Surg 18:1397, 2007
3. Costantino PD, Friedman CD, Shindo ML, et al: Experimental mandibular regrowth by distraction osteogenesis. Long-term results. Arch Otolaryngol Head Neck Surg 119:511, 1993
4. Costantino PD, Johnson CS, Friedman CD, et al: Bone regeneration within a human segmental mandible defect: A preliminary report. Am J Otolaryngol 16:56, 1995
5. Costantino PD, Shybut G, Friedman CD, et al: Segmental mandibular regeneration by distraction osteogenesis. An experimental study. Arch Otolaryngol Head Neck Surg 116: 535, 1990
6. González-García R, Naval-Gías L, Rubio-Bueno P, et al: Double-step transport osteogenesis in the reconstruction of mandibular segmental defects: A new surgical technique. Plast Reconstr Surg 118:1608, 2006
7. González-Garcia R, Rubio-Bueno P, Naval-Gías L, et al: Internal distraction osteogenesis in mandibular reconstruction: Clinical experience in 10 cases. Plast Reconstr Surg 121:563, 2008
8. Kuriakose MA, Shnayder Y, DeLacure MD: Reconstruction of segmental mandibular defects by distraction osteogenesis for mandibular reconstruction. Head Neck 25:816, 2003
9. Rubio-Bueno P, Padrón A, Villa E, et al: Distraction osteogenesis of the ascending ramus for mandibular hypoplasia using extraoral or intraoral devices: A report of 8 cases. J Oral Maxillofac Surg 58:593, 2000
10. Rubio-Bueno P, Sanromán F, García P, et al: Experimental mandibular regeneration by distraction osteogenesis with submerged devices: Preliminary results of a canine model. J Craniofac Surg 13:224, 2002
11. Cheung LK, Zhang Q, Zhang ZG, et al: Reconstruction of maxillectomy defect by transport distraction osteogenesis. Int J Oral Maxillofac Surg 32:515, 2003
12. Elsalanty ME, Zakhary I, Akeel S, et al: Reconstruction of canine mandibular bone defects using a bone transport reconstruction plate. Ann Plast Surg 63:441, 2009
13. Ashman RB, Cowin SC, Van Buskirk WC, et al: A continuous wave technique for the measurement of the elastic properties of cortical bone. J Biomech 17:349, 1984
14. Ashman RB, Van Buskirk WC: The elastic properties of a human mandible. Adv Dent Res 1:64, 1987
15. Dechow PC, Nail GA, Schwartz-Dabney CL, et al: Elastic properties of human supraorbital and mandibular bone. Am J Phys Anthropol 90:291, 1993
16. Schwartz-Dabney CL, Dechow PC: Variations in cortical material properties throughout the human dentate mandible. Am J Phys Anthropol 120:252, 2003
17. McKoy BE, Kang Q, An YH: Indentation testing of bone, *in* An YH, Draughn RA (eds): Mechanical Testing of Bone and Bone-Implant Interface. New York, CRC Press, 2000, p 233
18. Cano J, Campo J, Moreno LA, et al: Osteogenic alveolar distraction: A review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 101:11, 2006
19. Cope JB, Samchukov ML: Regenerate bone formation and remodeling during mandibular osteodistraction. Angle Orthod 70:99, 2000
20. Oda T, Sawaki Y, Ueda M: Experimental alveolar ridge augmentation by distraction osteogenesis using a simple device that permits secondary implant placement. Int J Oral Maxillofac Implants 15:95, 2000
21. Zhao Y: Bone healing process around distraction implants following alveolar distraction osteogenesis: A preliminary experimental study in dogs. Int J Periodontics Restorative Dent 29:523, 2009
22. Komisar A: The functional result of mandibular reconstruction. Laryngoscope 100:364, 1990
23. Kuriloff DB, Sullivan MJ: Mandibular reconstruction using vascularized bone grafts. Otolaryngol Clin North Am 24:1391, 1991
24. Takushima A, Harii K, Asato H, et al: Mandibular reconstruction using microvascular free flaps: A statistical analysis of 178 cases. Plast Reconstr Surg 108:1555, 2001
25. Herford AS: Use of a plate-guided distraction device for transport distraction osteogenesis of the mandible. J Oral Maxillofac Surg 62:412, 2004
26. Huh JY, Choi BH, Kim BY, et al: Critical size defect in the canine mandible. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 100:296, 2005
27. Toriumi DM, O'Grady K, Horlbeck DM, et al: Mandibular reconstruction using bone morphogenetic protein 2: Long-term follow-up in a canine model. Laryngoscope 109:1481, 1999
28. Kunz C, Adolphs N, Buscher P, et al: Mineralization and mechanical properties of the canine mandible distraction wound following acute molding. Int J Oral Maxillofac Surg 35:822, 2006
29. Ryoyama D, Sawaki Y, Ueda M: Experimental study of mechanical analysis in mandibular lengthening. Application of strain gauge measurement. Int J Oral Maxillofac Surg 33:294, 2004
30. Perrott DH, Rahn B, Wahl D, et al: Development of a mechanical testing system for a mandibular distraction wound. Int J Oral Maxillofac Surg 32:523, 2003
31. Mofid MM, Inoue N, Atabey A, et al: Callus stimulation in distraction osteogenesis. Plast Reconstr Surg 109:1621, 2002
32. Kaban LB, Thurmuller P, Troulis MJ, et al: Correlation of biomechanical stiffness with plain radiographic and ultrasound data in an experimental mandibular distraction wound. Int J Oral Maxillofac Surg 32:296, 2003
33. Hube R, Mayr H, Hein W, et al: Prediction of biomechanical stability after callus distraction by high resolution scanning acoustic microscopy. Ultrasound Med Biol 32: 1913, 2006
34. Costantino PD, Friedman CD, Shindo ML, et al: Experimental mandibular regrowth by distraction osteogenesis. Long-term results. Arch Otolaryngol Head Neck Surg 119:511, 1993
35. Farhadieh RD, Gianoutsos MP, Dickinson R, et al: Effect of distraction rate on biomechanical, mineralization, and histologic properties of an ovine mandible model. Plast Reconstr Surg 105:889, 2000
36. Gomez DF, Sant'Anna EF, Leven RM, et al: Microstructural and strength evaluation of regenerate tissue during the consolidation period after vertical mandibular ramus distraction. J Craniofac Surg 16:805, 2005
37. Ayoub AF, Richardson W, Koppel D, et al: Segmental mandibular reconstruction by microincremental automatic distraction osteogenesis: An animal study. Br J Oral Maxillofac Surg 39: 356, 2001
38. Ashman RB, Rosinia G, Cowin SC, et al: The bone tissue of the canine mandible is elastically isotropic. J Biomech 18:717, 1985
39. Ashman RB, Corin JD, Turner CH: Elastic properties of cancellous bone: Measurement by an ultrasonic technique. J Biomech 20:979, 1987
40. Schwartz-Dabney CL, Dechow PC: Edentulation alters material properties of cortical bone in the human mandible. J Dent Res 81:613, 2002
41. Cano J, Campo J, Gonzalo JC, et al: Consolidation period in alveolar distraction: A pilot histomorphometric study in the mandible of the beagle dog. Int J Oral Maxillofac Implants 21:380, 2006
42. Samchukov ML, Cope JB, Cherkasin AM: Biologic basis of new bone formation under the influence of tension stress, *in* Samchukov ML, Cope JB, Cherkashin AM (eds): Craniofacial Distraction Osteogenesis. Philadelphia, PA, Mosby, 2001, p 21

43. Weaver JK: The microscopic hardness of bone. J Bone Joint Surg [Am] 48:273, 1966
44. McKoy BE, Kang Q, An YH: Indentation testing of bone, *in* An YH, Draughn RA (eds): Mechanical Testing of Bone and Bone-Implant Interface. New York, CRC Press, 2000, p 233
45. Blackburn J, Hodgskinson R, Currey JD, et al: Mechanical properties of microcallus in human cancellous bone. J Orthop Res 10:237, 1992
46. Huja SS, Katona TR, Roberts WE: Microhardness testing of bone, *in* An YH, Draughn RA (eds): Mechanical Testing of Bone and Bone-Implant Interface. New York, CRC Press, 2000, p 247
47. Trebacz H, Zdunek A: Three-point bending and acoustic emission study of adult rat femora after immobilization and free remobilization. J Biomech 39:237, 2006
48. Johnson WM, Rapoff AJ: Microindentation in bone: Hardness variation with five independent variables. J Mater Sci Mater Med 18:591, 2007
49. Cope JB, Samchukov ML, Cherkashin AM: Mandibular distraction osteogenesis: A historic perspective and future directions. Am J Orthod Dentofac Orthop 115:448, 1999
50. Spagnoli D: Mandible reconstruction with transport distraction osteogenesis. Atlas Oral Maxillofac Surg Clin North Am 16:287, 2008
51. Aaron RK, Ciombor DM, Simon BJ: Treatment of nonunions with electric and electromagnetic fields. Clin Orthop Relat Res 419:21, 2004
52. Giotakis N, Narayan B, Nayagam S: Distraction osteogenesis and nonunion of the docking site: Is there an ideal treatment option? Injury 38:S100, 2007
53. Ilizarov GA: The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 238:249, 1989
54. Ilizarov GA: Tension-stress effect on the genesis and growth of tissues. Part II. The influence of the rate and frequency of distraction. Clin Orthop 250:8, 1990